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Piperine Inhibits Visceral Pain Caused by Acetic Acid in Mice

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Abstract: Piperine administered orally at increasing concentrations of 0.5-4 mg mL⁻¹ (5-40 mg kg⁻¹; 0.3 mL) caused dose-dependent inhibition of the number of abdominal constrictions induced 60 min later by i.p. injection of 0.6% acetic acid in mice by 14.5-66.2%. Higher concentrations of 8 or 16 mg mL⁻¹ (80-160 mg kg⁻¹; 0.3 mL) did not produce further inhibition of the nociceptive behavior (-49.5 and -33.9% inhibition, respectively). The inhibition of visceral pain by piperine was evident 15 min after its oral administration. The antinociceptive effect of orally administered piperine (4 mg mL⁻¹; 40 mg kg⁻¹) was unaffected by atropine (2 mg kg⁻¹, s.c.) or theophylline (20 mg kg⁻¹, s.c.), but increased by co-treatment with propranolol (2 mg kg⁻¹, s.c.), prazosin (2 mg kg⁻¹, s.c.), guanethidine (16 mg kg⁻¹, s.c.), glibenclamide (5 mg kg⁻¹, s.c.) or yohimbine (10 mg kg⁻¹, s.c.). Lidocaine administered orally just prior to piperine enhanced the antinociceptive effect of the latter. The antinociceptive effect of piperine (2 mg mL⁻¹) and dexamethasone (0.1 mg kg⁻¹, s.c.) or indomethacin (10 mg kg⁻¹, s.c.) was additive. The present study indicates that the oral administration of piperine exerts antinociceptive properties in a model of visceral inflammatory pain in mice. It is suggested that stimulation of sensory afferent by piperine and transmission of nociceptive information centrally leads to the activation of descending antinociceptive mechanism interfering with the noxious visceral stimulus.

Key words: Piperine, visceral pain, sensory afferent, mice

INTRODUCTION

Hot spices are widely used in human food due to their taste and burning sensations caused by their pungent principles. These are predominantly piperine (6%) in black pepper (*Piper nigrum*) and capsaicinoids in paprika (*Capsicum annum*: 0.01-0.22%), chili or cayenne pepper (*C. frutescens*: 0.3-1%) (Govindarajan, 1977). Apart from being used for food flavoring, the pungent capsaicin and piperine share important pharmacological properties. It has long been known that a subset of somatic and visceral primary afferent sensory neurons are excited by capsaicin, a vanillyl amide (8-methyl-N-vanillyl-6-nonenamide). These are peptidergic, small diameter neurons that give rise to unmyelinated C fibers or thinly myelinated A δ fibers, involved in nociception and neurogenic inflammation. They are polymodal and chemonociceptors that are responsive to noxious mechanical, thermal and chemical stimuli and convey nociceptive signals from the periphery to the spinal cord (Szolcsányi, 1977, 1984, 1990a, 1993; Holzer, 1991). The receptive properties of these sensory neurons are determined by their expression of transducing ion-channel receptors, which have a high threshold of activation to external stimuli. Nociceptors express the transient receptor potential vanilloid 1 (TRPV1) (formerly vanilloid

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receptor 1 or VR1) (Caterina and Julius, 2001), a member of the transient receptor potential (TRP) family of ion channels, a large group of proteins involved in the detection and integration of sensory stimuli (Clapham, 2003) and is directly activated by a wide range of stimuli including noxious heat, protons, endogenous lipoxygenase products and fatty acid amides as well as capsaicin, gingerols, eugenol, resiniferatoxin, piperine and camphor (Yang *et al.*, 2003; Dedov *et al.*, 2002; McNamara *et al.*, 2005).

TRPV1 is expressed in most of the afferent nerve fibres in the rodent gastrointestinal tract and some of the vagal afferent and plays a role in neurogenic inflammation and visceral pain. For example, vanilloid-mediated TRPV1 refractoriness is effective in visceral hypersensitivity. In this context, the capsaicin-analogues resiniferotoxin and SDZ249-665 effectively attenuated inflammatory bladder hyperalgesia (Dinis *et al.*, 2004) and visceral pain responses to intraperitoneal acetic acid in animals (Jaggar *et al.*, 2001).

Black pepper, is a perennial plant, belongs to the *Piperaceae* family, *Piper* genus. The fruit becomes black when dried, with strong aroma and hot flavor. Black pepper is the table spice that is widely used to flavor food. It is estimated that the average daily consumption of black pepper per person in the U.S. is 446 mg and that of the pungent piperine is 21 mg (Gold *et al.*, 2001). In view of the ability of piperine (1-peperoylpiperidine), the primary pungent alkaloid found in black pepper to activate TRPV1 (McNamara *et al.*, 2005) and in view of the role of TRPV1 in gut sensation (Holzer, 2004a, b), it therefore looked pertinent to see the effect of oral administration piperine on visceral pain. The acetic acid-induced writhing test in mice was used for this purpose, where the intraperitoneal (i.p.) injection of acetic acid to rats triggers abdominal contractions as a manifestation of pain, which is used for quantifying visceral pain with inflammation (Koster *et al.*, 1959).

MATERIALS AND METHODS

Animals

Swiss male albino mice 25-30 g of body weight were used. Standard laboratory food and water were provided *ad libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Equal groups of 6 mice each were used in all experiments.

Acetic Acid-Induced Writhing

Separate groups of 6 mice each were administered vehicle (distilled water) or piperine 0.5-16 mg mL⁻¹ (5-160 mg kg⁻¹; 0.3 mL, p.o.). After 60 min pretreatment interval, an i.p. injection of 0.6% acetic acid (0.2 mL) was administered (Koster *et al.*, 1959). The effect of piperine 4-16 mg mL⁻¹ (40-160 mg kg⁻¹; 0.3 mL, p.o.) administered 15 min prior to acetic acid challenge was also studied. Furthermore, in another series of experiments, the effects of piperine (4 mg mL⁻¹; 40 mg kg⁻¹, 0.3 mL p.o.) on anti-writhing induced by indomethacin (10 mg kg⁻¹, s.c.) or dexamethasone (0.1 mg kg⁻¹, s.c.) was examined. Drugs were administered 60 min prior to the abdominal constriction assay.

Further experiments were designed in an attempt to elucidate the mechanisms by which piperine exerts its anti-nociceptive effect. Piperine at concentration of 4 mg mL⁻¹; 40 mg kg⁻¹, 0.3 mL, p.o.) was selected to be used in the subsequent experiments and administered 60 min prior to nociceptive challenge with i.p. acetic acid. Thus, the effect of co-administration of the alpha-1 adrenoceptor antagonist prazosin (2 mg kg⁻¹, s.c.), the alpha-2 adrenoceptor antagonist yohimbine (5 or 10 mg kg⁻¹, s.c.), the beta adrenoceptor antagonist, propranolol (2 mg kg⁻¹, s.c.), the adrenergic

blocker, guanethidine (16 mg kg⁻¹, s.c.), the muscarinic acetylcholine receptor antagonist atropine (2 mg kg⁻¹, s.c.), the non-selective adenosine receptor antagonist theophylline (20 mg kg⁻¹, s.c.) and the potassium channel blocker glibenclamide (5 mg kg⁻¹, s.c.) were examined on antinociception caused by piperine. In addition, the effect of the local anaesthetic lidocaine (0.1 mL of 2% solution, p.o.) given just prior to piperine was studied.

Drugs and Chemicals

Piperine, atropine sulfate, yohimbine hydrochloride, propranolol hydrochloride, guanethidine hydrochloride (Sigma, St. Louis, USA) were used. Analytical-grade glacial acetic acid (Sigma, St. Louis, USA) was diluted with pyrogen-free saline to provide a 0.6% solution for i.p. injection. All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use.

Statistical Analyses

Data are expressed as mean±SE. Data were analyzed by one way analysis of variance, followed by a Tukey's multiple range test for *post hoc* comparison of group means. A probability of $p < 0.05$ were considered to be significant.

RESULTS

Antinociceptive Effect of Piperine

Piperine administered orally at concentrations of 0.5, 1, 2 or 4 mg mL⁻¹ (5, 10, 20 or 40 mg kg⁻¹; 0.3 mL) caused dose-dependent inhibition of abdominal constrictions induced 60 min later by i.p. acetic acid injection in mice by 14.5, 30.4, 53.7 and 66.2%, respectively. Higher concentrations of 8 or 16 mg mL⁻¹ (80 or 160 mg kg⁻¹; 0.3 mL) did not produce further inhibition of the nociceptive behavior (-49.5 and -33.9% inhibition, respectively) (Fig. 1). The inhibition of visceral pain by piperine was evident 15 min after its oral administration. Piperine administered orally at concentrations of 4, 8 or 16 mg mL⁻¹ (40, 80 or 160 mg kg⁻¹; 0.3 mL) 15 min before acetic acid challenge, reduced the number of abdominal constrictions by 67.4, 30.7, 29.4%, respectively (Fig. 2). The antinociceptive effect of piperine (2 mg mL⁻¹) and dexamethasone (0.1 mg kg⁻¹, s.c.) or indomethacin (10 mg kg⁻¹, s.c.) was additive (Fig. 3).

Effect of Propranolol or Atropine

The antinociceptive effect of orally administered piperine (4 mg mL⁻¹; 40 mg kg⁻¹) was unaffected by atropine (2 mg kg⁻¹, s.c.), but enhanced by the non-selective beta adrenoreceptor antagonist propranolol (2 mg kg⁻¹, s.c.) (Fig. 4).

Effect of Prazosin, Yohimbine or Guanethidine

Figure 5 shows that co-treatment with the alpha (1) adrenoreceptor prazosin (2 mg kg⁻¹, s.c.), the alpha (2)-adrenoreceptor antagonist yohimbine (10 mg kg⁻¹, s.c.) or adrenergic neurone blocker guanethidine (16 mg kg⁻¹, s.c.) enhanced the antinociceptive effect of orally administered piperine (4 mg mL⁻¹; 40 mg kg⁻¹, 0.3 mL).

Effect of Theophylline or Glibenclamide

The antinociceptive effect of orally administered piperine (4 mg mL⁻¹; 40 mg kg⁻¹) was unaffected by co-treatment with the non-selective adenosine receptor antagonist theophylline (20 mg kg⁻¹, s.c.), but enhanced by the potassium channel blocker glibenclamide (5 mg kg⁻¹, s.c.) (Fig. 5).

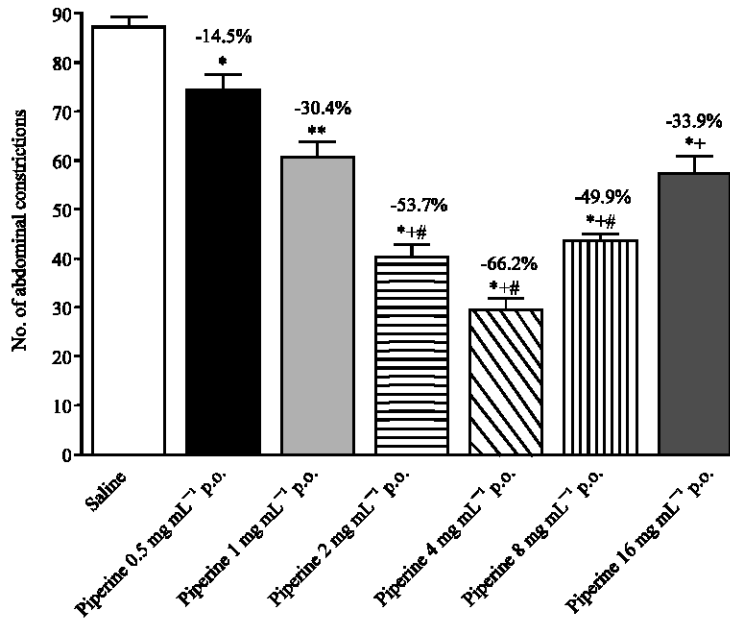


Fig. 1: Effect of piperine administered orally on abdominal constrictions caused by i.p. injection of acetic acid in mice. Piperine at concentrations of 0.5, 1, 2, 4, 8 or 16 mg mL⁻¹ (5, 10, 20, 40, 80 or 160 mg kg⁻¹; 0.3 mL) or vehicle (saline) was administered 60 min prior to acetic acid. Data represent mean±SE and percent inhibition (%) compared to the saline-treated group. *p<0.05 compared to saline. +p<0.05 vs piperine 0.5 mg mL⁻¹-treated group. #p<0.05 vs piperine 1 or 16 mg mL⁻¹-treated group

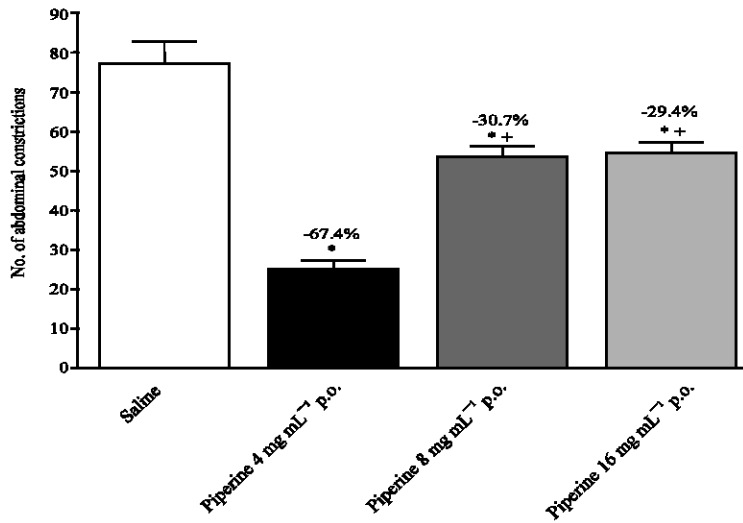


Fig. 2: Effect of piperine administered orally 15 min prior to i.p. injection of acetic acid in mice. Piperine was given at concentrations of 4, 8 or 16 mg mL⁻¹ (40, 80 or 160 mg kg⁻¹; 0.3 mL). Data represent mean±SE and percent inhibition (%) compared to the saline-treated group. *p<0.05 compared to saline. +p<0.05 vs piperine 4 mg mL⁻¹ treated group

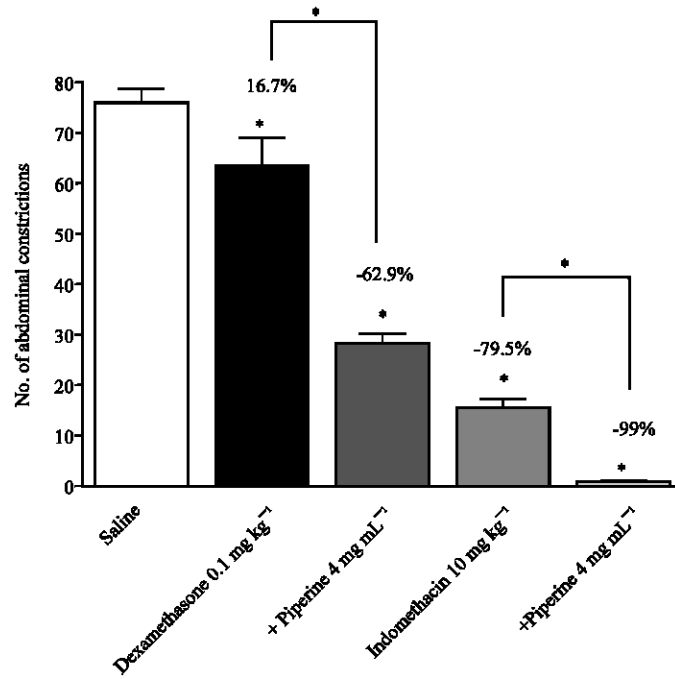


Fig. 3: Effect of piperine administered orally on antinociception caused by dexamethasone (0.1 mg kg⁻¹, s.c.) or indomethacin (10 mg kg⁻¹, s.c.) in the abdominal constriction assay in mice. Drugs were administered 60 min prior to i.p. injection of acetic acid. Data represent mean±SE and percent inhibition (%) compared to the saline-treated group. *p<0.05 compared to saline and between different groups as shown in the figure

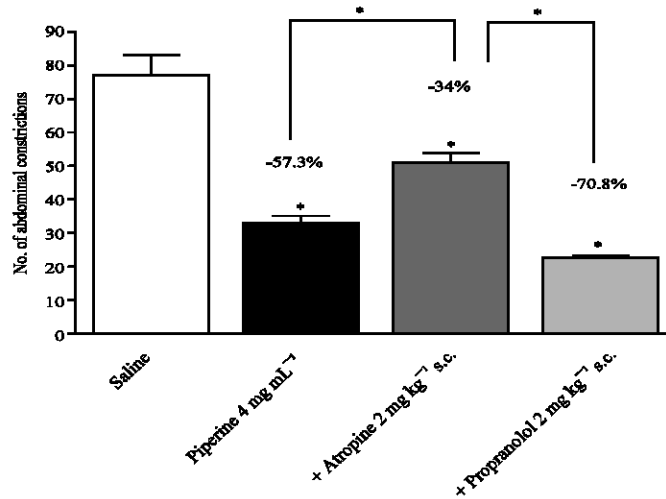


Fig. 4: Effect of atropine (2 mg kg⁻¹, s.c.) or propranolol (2 mg kg⁻¹, s.c.) on visceral antinociception induced by orally administered piperine (4 mg mL⁻¹, 0.3 mL) in the abdominal constriction assay. Drugs were administered 60 min prior to the test. Data represent mean±SE and percent inhibition (%) compared to the saline-treated group. *p<0.05 compared to saline and between different groups as shown in the figure

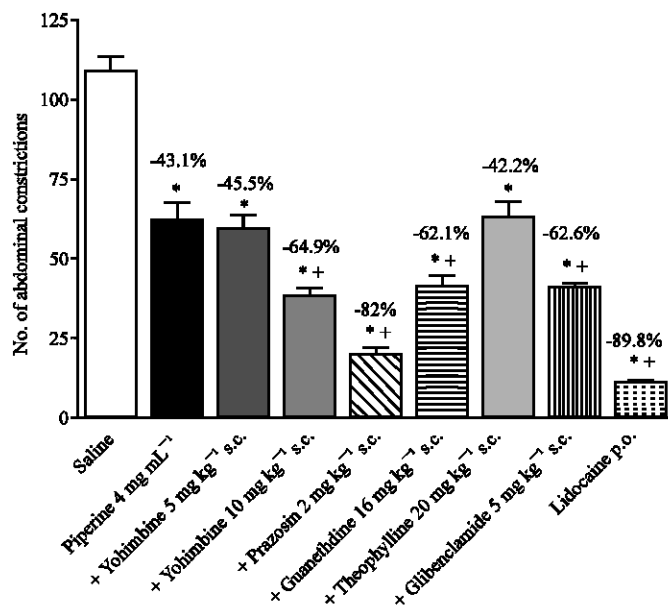


Fig. 5: Effect of prazosin (2 mg kg⁻¹, s.c.), yohimbine (5 or 10 mg kg⁻¹, s.c.), guanethidine (16 mg kg⁻¹, s.c.), theophylline (20 mg kg⁻¹, s.c.), glibenclamide (5 mg kg⁻¹, s.c.) or lidocaine (0.1 mL of 2% solution, p.o.) on visceral antinociception induced by orally administered piperine (4 mg mL⁻¹, 0.3 mL) in the abdominal constriction assay. Drugs were administered 60 min prior to the test. Data represent mean±SE and percent inhibition (%) compared to the saline-treated group. *p<0.05 compared to saline. +p<0.05 vs piperine alone or black pepper + yohimbine (5 mg kg⁻¹) or theophylline. Those treated with piperine and either prazosin or lidocaine exhibited significantly less abdominal constrictions than all other groups

Effect of Lidocaine

Lidocaine administered orally just prior to piperine enhanced the antinociceptive effect of the latter (Fig. 5).

DISCUSSION

Piperine, the active ingredient of black peppers, a widely consumed table spice and food flavoring agent is shown in the present study to reduce the number of abdominal constrictions evoked by i.p. acetic acid in mice. It is also evident that the observed reduction in nociceptive behavior following piperine was neither due to sympathetic or cholinergic reflexes and is unlikely to involve adenosine receptors or ATP-gated potassium channels. These results are in accordance with the specific site of action for piperine on capsaicin sensitive sensory nerves. The present findings are the first which describe the effect of piperine on visceral inflammatory pain in experimental animals.

The present observation is intriguing since piperine similar to capsaicin is a stimulant of unmyelinated C fibers or thinly myelinated A δ fibers (capsaicin-sensitive sensory nerves) that gives rise to polymodal and chemoreceptors which are responsive to noxious thermal and chemical stimuli and convey nociceptive signals from the periphery to the spinal cord (Szolcsányi, 1977, 1984; Holzer, 1991). In other words, the present findings suggest that excitation of this special subset of sensory nerves (capsaicin-sensitive sensory nerves) prevented manifestations of pain i.e., abdominal contractions evoked by chemical peritoneal irritation.

Visceral pain is a poorly understood type of pain. In contrast to somatic pain which can be precisely localized, the perception of visceral pain is ill defined, possibly reflecting differences in the pattern of somatic and visceral input to the cerebral cortex and is often referred to somatic structures distant from the site of inflammation which may be explained by the central convergence of visceral and somatic inputs (Ness and Gebhart, 1990; Cervero and Laird, 1999). One commonly used experimental model to study the pathogenetic mechanisms of visceral pain involves the injection into the peritoneal cavity of mice of dilute acetic acid. This type of visceral inflammatory type of pain is brought about by the formation of prostaglandins which in turn release calcitonin-gene related peptide (CGRP) from afferent nerve fibres and give rise to abdominal muscle contractions, a reaction indicative of pain (Berkenkopf and Weichman, 1988; Friese *et al.*, 1997).

The precise mechanism or site of action whereby piperine exerts its analgesic effect is not clear. Pain processing involves nociception or the detection of noxious stimuli and the subsequent transmission of encoded information to the brain. Capsaicin-sensitive sensory nerves can modulate visceral sensation as they convey signals coming from the gastrointestinal tract to the central nervous system and may simultaneously release transmitters able to affect local tissue functions (Holzer, 1991, 2004a, b). In this context, increased expression in subcortical nuclei and spinal cord of c-fos, a marker for activity following noxious somatic or visceral stimulation was evoked by intragastric capsaicin (3.2 mM; 2 mL), intragastric HCl (0.5 M) (Michl *et al.*, 2001) and by noxious gastric distention (Traub *et al.*, 1996). The nociceptive information being processed both by gastric vagal and intestinal spinal afferent (Traub *et al.*, 1996; Holzer *et al.*, 2005). This suggests roles for these brain stem regions in mediating sensory and reflex responses to irritant chemical stimulation of the upper gastrointestinal mucosa (Michl *et al.*, 2001; Holzer *et al.*, 2005).

Modulation of the sensory input can occur within the dorsal horn of the spinal cord where the primary afferent fibers synapse with neurons that transmit to the higher centers. In this context, noxious gastric stimulation with acid (0.5 M HCl) induced the release of glutamate, SP and CGRP from capsaicin-sensitive sensory afferent in the dorsal horn of the spinal cord where they may play an important role in gastric nociception and hyperalgesia (Schicho *et al.*, 2005). Evidence also indicates the existence of a descending modulation of pain, whereby, powerful inhibitory influences arising from the brain descends in the spinal cord to modulate spinal visceral nociceptive transmission. Thus, electrical and/or chemical (glutamate) stimulation of periaqueductal gray or rostral ventromedial medulla (Giesler and Liebeskind, 1976; Ness and Gebhart, 1987; Zhuo *et al.*, 2002) or thalamic nucleus submedius (Yang and Follett, 2003) attenuated the neuronal responses to a noxious visceral stimulus (colorectal distension).

Nociceptive stimulation also results in a neurogenic inflammatory response, with the release of compounds such as substance P, neurokinin A and CGRP from the peripheral terminals of nociceptive afferent fibers. The administration of CGRP mimicked the effects of acetic acid (Julia and Bueno, 1997) and increased the number of abdominal contractions in response to colorectal distension in rats (Plourde *et al.*, 1997). These effects were reversed by intravenous administration of CGRP antagonist (hCGRP-(8-37) or by systemic capsaicin pretreatment which depletes sensory nerves of their neuropeptide content (Julia and Bueno, 1997; Plourde *et al.*, 1997).

Piperine has been shown to affect gastrointestinal functions. Piperine applied into the stomach in very low concentrations was able to protect the gastric mucosa of the rat against ethanol-induced injury. These effects are mediated through the release of vasodilator peptides from capsaicin-sensitive sensory nerve endings upon their stimulation with the pungent piperine (Szolcsányi, 1990b). Piperine (0.5 or 20 mg kg⁻¹ i.p.) dose-dependently delayed gastrointestinal motility (Izzo *et al.*, 2001).

It is not clear whether the effect of piperine on visceral pain reflects a central or peripheral action. Limited data is available as regards the absorption and metabolism of piperine. In rats, it is likely that piperine is well absorbed following ingestion, the highest concentration in the stomach and small

intestine being attained at about 6 h. Only piperine was detected in the serosal fluid and the intestinal tissue, indicating that piperine did not undergo any metabolic change during absorption (Bhat and Chandrasekhara, 1986). Piperine may thus be absorbed and reach the intestine or cross the blood brain barrier to exert a pain modulating effect. Since, however, the effect of piperine was evident as early as 15 min of oral administration, it remains to be determined whether piperine could be absorbed in sufficient amount so as to reach the gastrointestinal tract or penetrate the blood brain barrier. Another yet an intriguing possibility would be that stimulation of sensory afferent by piperine and transmission of nociceptive information centrally leads to the activation of descending antinociceptive mechanism to the noxious visceral stimulus.

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